

### IN THE SPECIFICATION:

Please replace the first full paragraph on page 2, which starts "In recent years" with the following:

In recent years PCR based diagnostic methods have been described for leishmaniasis, with a wide range of sensitivity and specificity. An excellent target for a sensitive and rapid detection method is the kinetoplast mini-circle DNA, which are present at thousands of copies per cell. The mini-circles have been used as targets for selective amplification of parasite DNA in various studies { Aviles, H., A. Belli, R. Armijos, F.P. Monroy, and E. Harris J. Parasitol. 1999, 85:181-187; Bhattacharya R., K. Das, S. Sen, S. Roy, and H.K. Majumder. 1996. Microbiol. Lett. 135:195-200; Nuzum, E., F. White III, C. Thakur, R. Dietze, J. Wages, M. Grogl, and J. Berman. 1995 J. Inf. Dis. 171: 751-754; Rogers M.R, Popper S.J., and Wirth D.F. 1990. Exp. Parasitol. 71: 267-275; Smyth, A.J., A. Ghosh, Md. Q. Hassan, D. Basu, M.H.L. De Bruijn, S. Adhya K.K. Mallik, and D.C. Barker. 1992, Parasitol 105: 183-192.}

Please replace the first full paragraph on page 5, which starts "The primers developed" with the following:

The primers developed are 5'-AAATCGGCTCCGAGGCGGGAAAC-3' and 5'-GGTACACTCTATCAGTAGGAC-3' both together designated as Ldl primers or ~~SEQ ID Nos.~~ SEQ ID NOS: 1 and 2 respectively. These primers have been developed after analysis of the 792 bp *L. donovani* kinetoplast minicircle sequence deposited at [www.ncbi.nlm.nih.gov/genbank](http://www.ncbi.nlm.nih.gov/genbank) at Accession No. Y11401. The web site provides

more than 245 entries for leishmanial kDNA, all of which have been ~~analysed~~ analyzed by this Applicant. It is after such detailed analysis that the primers of the invention were developed.

Please replace the first full paragraph from the bottom on page 5, which starts "In the present" with the following:

In the present invention, the primers were synthesized as described above using the said Applied Biosystems DNA/RNA synthesizer model 394. The DNA from the clinical samples was amplified using the primers of the invention i.e. ~~SEQ ID No.~~ SEQ ID NO: 1 and ~~SEQ ID No.~~ SEQ ID NO: 2. The PCR products were ~~analysed~~ analyzed by gel electrophoresis. The PCR products were subjected to southern blot analysis and hybridized with <sup>32</sup>P labeled cloned *Leishmania donovani* kDNA fragment (kinetoplast DNA). The PCR products were cloned in an appropriate vector system, sequenced and analysed using PC-Gene software to arrive at the said novel primers.

Please replace the paragraph bridging pages 5 and 6, which starts "Thus, the invention" with the following:

Thus, the invention provides a PCR primer set specific for *Leishmania donovani*, said primer set being (1) a first pair of oligonucleotides having the sequences given by ~~SEQ ID No.~~ SEQ ID NO: 1 and ~~SEQ ID No.~~ SEQ ID NO: 2 wherein the primer set is effective in a PCR assay for detecting the presence of ~~*Leishmania donovani*~~ infection in samples derived from patients infected by leishmaniasis. The primer set is a first pair of oligonucleotides. ~~SEQ ID No.~~ SEQ ID NO: 1 is 5'-

AAATCGGCTCCGAGGCGGGAAAC-3' and ~~SEQ ID No~~ SEQ ID NO: 2 is 5'-GGTACACTCTATCAGTAGCAC-3'.

Please replace the first full paragraph on page 6, which starts "Further, the invention" with the following:

Further, the invention provides a method of detecting the presence of *Leishmania donovani* in a sample from a patient suspected of leishmaniasis, said method comprising the steps of:

- 1) providing a sample from the patient suspected fo being infected with *Leishmania donovani*
  - 2) isolating and purifying the nucleic acids from the sample,
  - 3) forming a polymerase chain reaction solution containing at least a portion of nucleic acids form step (b), a PCR primer set consisting of ~~SEQ ID Nos:~~ SEQ ID NOS: 1 and 2, a mixture of nucleoside triphosphate monomers, and an enzyme *Taq* polymerase in a buffered solution,
  - 4) carrying out a polymerase chain reaction on the PCR reaction solution to amplify any *Leishmania donovani*-specific nucleic acid; and
  - 5) ~~analysing~~ analyzing the *Leishmania donovani*-specific nucleic acids obtained in the polymerase chain reaction using gel-electrophoresis method and staining the resulting gel,
- wherein the presence of a band at about 600bp is indicative of the presence of *Leishmania donovani parasites* in the patient.

Please replace the first paragraph on page 7, which starts "In addition," with the following:

In addition, the invention provides a kit for detecting *Leishmania donovani* in a sample, comprising oligonucleotide primers, wherein the primers comprise ~~SEQ ID~~ SEQ ID NO: 1 and ~~SEQ ID NO: 2~~ SEQ ID NO: 2, and wherein the primers hybridize to the said *Leishmania donovani*.

Please replace the paragraph bridging pages 12 and 13, which starts "A total of " with the following:

A total of 107 clinical samples from leishmaniasis patients were examined and 95% tested positive in PCR. The PCR described in this invention yielded a unique product of 600bp and no non-specific side product or artifacts appeared on the gel. It has the advantage that results were easily and unequivocally interpreted upon analysis on agarose gels. The high level of sensitivity was reflected by the ability of the assay to detect parasite DNA in peripheral blood of KA patients with 96% sensitivity in the 51 cases examined. Use of peripheral blood is advantageous because the collection procedure is less invasive and safer than the splenic or bone marrow biopsy specimen collection. In earlier studies for diagnosis of VL due to *L. donovani*, the sensitivity of PCR for blood samples has been found to be in the range of 45-94% based on smaller sample size ranging from 17 to 42. [ Adhya, S., M. Chatterjee, et al Trans. R. Soc. Trop. Med. Hyg. 1995; 89: 622-624, Andresen, K., S. Gasim, A.M. et al 1997, 2: 440-444; Katakura, K., S.I. Kawazu, T. Naya, et al 1998. J. Clin. Microbiol. 36:2173-2177; Nuzum, E., F. White III, et al. J. Inf. Dis. 171: 751-754; Osman, O.F., L.

Oskam, et al J. Clin. Microbiol. 1977, 35:2454-2457; +++Singh N., M.D. Curran, et al Trop. Med & Int. Health. 1999, 4;448-453; Smyth, A.J., A. Ghosh, et al 1992. Parasitol 105: 183-192.}

Please replace the first full paragraph on page 13, which starts "For detection of VL" with the following:

For detection of VL due to *L. infantum*, which may have a different pathogenesis, sensitivities between 64-97% have been reported with blood samples {Lachaud, L., J. Dereure, et al 2000. J. Clin. Microbiol. 38:236-240; Mathis, A., and P. Deplazes. 1995. J. Clin. Microbiol, 33:1145-1149; Nuzum, E., F. White III, et al 1995. J. Inf. Dis. 171: 751-754}. The sensitivity of detection was cent percent in the limited number of bone marrow samples that we examined. Bone marrow is known to have a high load of parasites while in peripheral blood the parasites are relatively scarce. Studies reporting PCR with detection sensitivity comparable to ours (less than a single parasite) did not obtain sensitivity as high as our assay when using blood samples of KA patients {Katakura, et al 1998. J. Clin. Microbiol. 36:2713-2177; Smyth, A.J., A. Ghosh et al 1992 Parasitol 105: 183-192}.

Please replace the paragraph bridging pages 13 and 14, which starts "DNA isolated from" with the following:

DNA isolated from the pathogens causative of common co-endemic diseases (*M. leprae*, *M. tuberculosis* and *Plasmodium*) was not amplified. Blood from malaria and tuberculosis patients were PCR negative in all cases (30/30) while two of the endemic

controls were PCR positive, giving an overall specificity of 96% in the control blood samples examined. The two positive endemic controls were relatives of KA patients and possibly asymptomatic carriers since both cases reacted positive in ELISA with recombinant antigen k39 and in dipstick test using immunochromatographic strips coated with rk39 antigen (Salotra and Sreenivas, unpublished data), tests reported to be specific for KA. [Singh, S., A.G. Sachs, et al 1995 J. Parasitol 81:1000-1003; Smyth, A.J., A. Ghosh, et al 1992. Parasitol 105: 183-192; Sundar, S., S.G. Reed, et al 1998. Lancet. 351:563-565]. A recent study has reported a PCR assay that could often detect parasitemia a few weeks before the appearance of any clinical signs or symptoms [Lachaud, L., J. Dereure, et al 2000. J. Clin. Microbiol. 38:236-240].